

THE ULTRAVIOLET ABSORPTION OF
PROTEIN-BOUND GLUTARALDEHYDE*

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In the course of our studies on the interaction between aldehydes and hide substance, which is principally collagen, glutaraldehyde emerged as a versatile new tanning agent which has attained some measure of commercial use in tanning of leather (1, 2). Other investigators have demonstrated the use of glutaraldehyde as a fixative for tissue studies (3), for hardening of gelatin (4, 5), for hardening of a synthetic sausage casing from fibrous animal materials (6), and as a reagent for stabilization of wool (7). Thus glutaraldehyde has been shown to be a versatile protein reagent. In fact, some authors believe that glutaraldehyde is a more versatile protein reagent than formaldehyde, at least in respect to its reactivity toward collagen (1).

A direct method for demonstrating the presence of protein-bound glutaraldehyde was non-existent and data on its fixation were by necessity obtainable only by indirect means. However, from the standpoint of analysis of the glutaraldehyde-treated proteins and elucidation of the mechanism of interaction, it was important to develop a direct method for the determination of bound glutaraldehyde. This communication describes a spectral method for demonstrating the presence of protein-bound glutaraldehyde, which depends on absorbance at 265 m μ .

Attempts in our laboratory to liberate the aldehyde from glutaraldehyde-tanned collagen, even by hydrolysis of the latter for 16 hours in refluxing 6N HCl, failed to produce any aldehyde whatsoever on the basis of a test with 2,4-dinitrophenylhydrazine reagent. This indicated the irreversible nature of the fixation of glutaraldehyde in contrast to other aldehydes, notably formaldehyde (8), acrolein (9), and crotonaldehyde (9), which are quantitatively estimated after hydrolysis of leathers tanned with these aldehydes.

Although the hydrolyzate of glutaraldehyde-tanned collagen failed to show the presence of an aldehyde, examination of the ultraviolet absorption spectrum of this hydrolyzate revealed the presence of some unknown substance showing a strong absorption maximum. The peak occurred in the mid-ultraviolet region of 2500 to 3200 Å which is ordinarily ascribed to the presence of aromatic amino acids in natural proteins. These, namely, tyrosine, tryptophane, and phenylalanine

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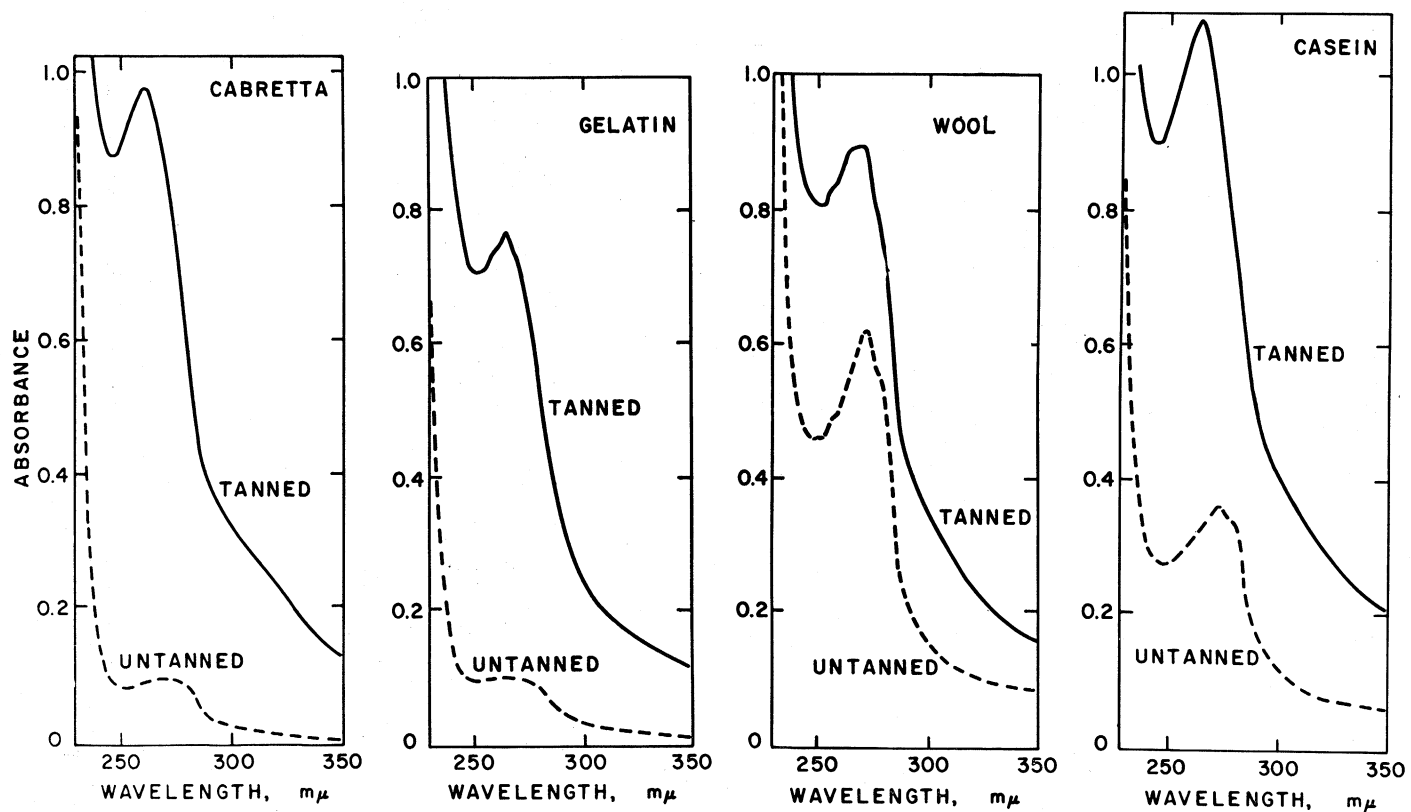


FIGURE 1.—Ultraviolet spectra of hydrolyzates of glutaraldehyde-tanned proteins. The hydrolyzates from 2 g. of air-dried protein were diluted to a concentration of 0.8 mg. protein/ml. in the cases of collagen, gelatin, and wool, and to a concentration of 0.4 mg. protein/ml. for casein.

(10, 11), are present only in very small amounts in collagen (12) and contribute little absorption, as indicated in the blank (Figure 1). The glutaraldehyde-tanned collagen, however, showed a sharp peak at 265 $m\mu$ that correlates with fixation of glutaraldehyde and is a convenient and direct method for demonstrating this fixation (see Figure 1). Hydrolyzates of formaldehyde-, glyoxal-, and α -hydroxyadipaldehyde-tanned collagen were devoid of this sharp absorption maximum at 265 $m\mu$ and showed only the weak absorbance in this region characteristic of hydrolyzates of collagen itself.

An absorption maximum at about 265 $m\mu$ was also noted in hydrolyzates of other proteins treated with glutaraldehyde. Gelatin was essentially the same as collagen. Hydrolyzates of glutaraldehyde-treated casein and wool gave a somewhat broadened peak. This undoubtedly reflects the contribution to the spectrum of the aromatic amino acids which absorb at about 275 $m\mu$ and are present in relatively large amounts in these proteins (see Figure 1). Thus, spectral analysis offers considerable promise in studies on the protein-glutaraldehyde interaction.

The collagen used in this study was in the form of degreased, pickled cabretta skins obtained from a tannery. The wool was obtained by clipping from a scoured, pickled shearling (woolskin). The gelatin was calfskin gelatin, type 1487, supplied by the Kind and Knox Gelatin Corporation.‡ The casein was an acid-precipitated product available commercially.

These proteins, which were insoluble under the conditions of treatment, were tanned with glutaraldehyde (12.5 percent on the dry weight of protein) at a pH of about 8 by procedures described previously (1, 2). The unbound glutaraldehyde was then completely removed by washing the treated proteins with water, and the tanned protein dried at ambient room conditions. Two grams of the air-dried protein (moisture content 7.75 to 12.3 percent) were hydrolyzed by refluxing for at least 2 hours with 15 ml. of 6*N* HCl. The hydrolyzate was evaporated to dryness on a steam bath and the residue dissolved in water. The solution was made up to 50 ml., filtered, and diluted appropriately with water before analyzing with a Cary Model 14 recording spectrophotometer, using a 10 mm. path absorption cell. The absorption spectra of the hydrolyzates of the glutaraldehyde-treated proteins and of the blanks (untreated protein) are shown in Figure 1. Gelatin and collagen, as expected, were comparable to each other, whereas casein closely resembled wool.

‡The mention of brand or firm names does not constitute an endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

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